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Reparation at Increased Oxygen Supply¹

The increase of the oxygen concentration in the breathing gas of the rat to about 50% v/v enhances the tensile strength of the healing skin wound by 40%². An augmented synthesis of collagen in vitro has been reported also as the effect of the increased oxygen in the gas phase³. This effect could not depend entirely on the requirement of oxygen for the hydroxylation of proline in protocollagen, because the K_m is only about 2.6% v/v⁴. The present experiments show that the increased oxygen supply affects also components other than collagen in the regenerating tissue.

For the experiments with ³H-proline (Figure, a and b), 4 viscose cellulose-sponges (1 × 1 × 2 cm) were implanted s.c. in the backs of male Wistar rats (weight 200 ± 30 g). The animals were closed into boxes where they breathed either air or 40% v/v oxygen. After 10 days the rats were decapitated, samples pooled from 4 sliced granulomas and incubated in 20 ml of Krebs-Ringer phosphate medium with 22.4 mM glucose. The gas phase was either air or 40% v/v oxygen. After a preincubation period of 30 min 80 μCi of ³H-proline (The Radiochemical Centre, Amersham, England) were added and the incubation continued for 4 h. The tissue was washed twice with ice-cold water after separating it from the medium by centrifugation at 0°C and 30,000 g for 10 min. The material was homogenized in water, hydrolyzed and the total activity and radioactivity of hydroxyproline were determined^{5,6} using Packard Tri-Carb 3320 liquid scintillation spectrometer (Packard Instrument Company Inc., Downers Grove,

Illinois, USA). When ³H-proline was used as a label, only 3–10% of the incorporated ³H was recovered in ³H-hydroxyproline. Because proline and hydroxyproline occur in collagen in approximately equal amounts, the radioactivity of collagen was accepted to be twice that of hydroxyproline. The radioactivity of non-collagenous protein was obtained by subtracting the ³H-activity of collagen from the total ³H-activity.

The experiments with ³H-cytidine (Figure, c) were performed analogously except that 30 μCi of the precursor was added. The incubation was stopped by the addition of cold perchloric acid solution to the final concentration of 0.5 N. The isolation of RNA was based on hydrolysis in 0.3 N potassium hydroxide at +37°C for 20 h and subsequent neutralization at 0°C with perchloric acid which

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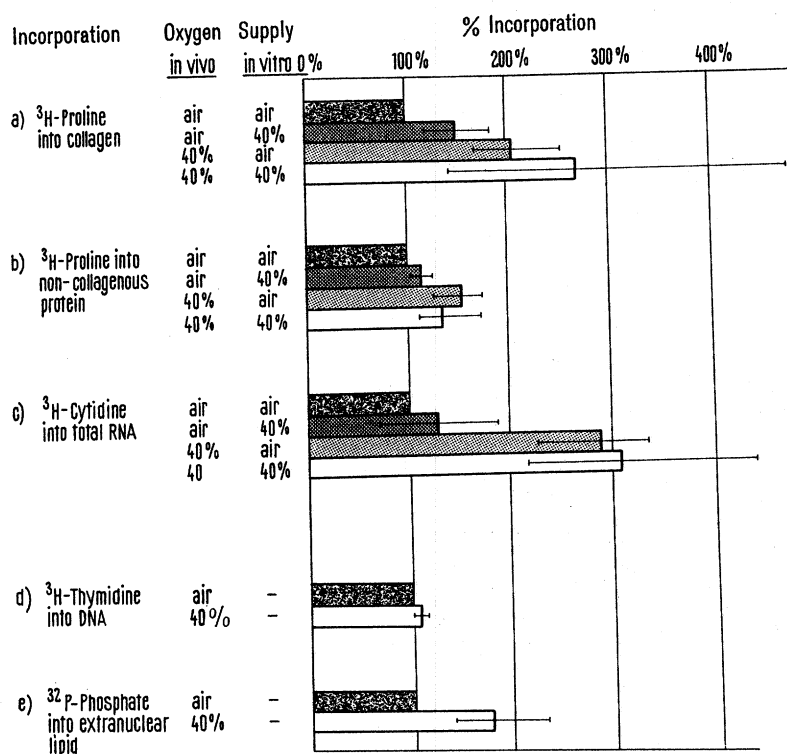
² J. NIINIKOSKI, R. PENTTINEN and E. KULONEN, *Acta physiol. scand. suppl.* 277, 146 (1966). – E. KULONEN, J. NIINIKOSKI and R. PENTTINEN, *Acta physiol. scand.* 70, 112 (1967). – J. NIINIKOSKI, *Acta physiol. scand. suppl.* 334, 72 pp. (1969).

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Incorporation of labelled precursors into the components of experimental granulation-tissue grown in rats which were kept at various oxygen tensions. The slices from the granulomas were then incubated with the gas phases indicated (a-c). The results which had been obtained supplying air during both the periods are taken as 100%. The given averages and ranges are based on 3 independent experiments.

was again added in excess to the final concentration of 0.5 N. The supernatant was used for the analysis of RNA-nucleotides.

The experiments with ³H-thymidine and ³²P-phosphate (Figure, d and e) were carried out entirely in vivo. ³H-thymidine (100 µCi in 1.0 ml of 0.9% NaCl solution) was injected i.p. to sponge-bearing rats on the 8th and 9th post-implantation days and the sponge-implants were removed on the 10th day. For the analysis of DNA the granulomas were first treated as for the determination of RNA, but DNA in the perchloric acid-precipitate was hydrolyzed by heating in 0.5 N perchloric acid at +70 °C for 30 min. The DNA-derived nucleotides remained in the supernatant obtained by centrifugation at 20,000 g for 30 min.

For the incorporation of ³²P-phosphate into lipids 1 mCi was injected i.p. The sponge-pieces were removed after 1-19 h incorporation on the 10th post-implantation day. In Figure e, the interval is 255 min. 2 granulomas were taken from the same side of the rat, homogenized at 0 °C and 20,000 rpm (Omni-Mixer®, type OM, Ivan Sorvall Inc., Norwalk, Connecticut, USA) for 2 min in 24 ml of chloroform-methanol (1:1, v/v). To remove the nuclei, the homogenate was centrifuged at +4 °C and 600 g for 15 min. The sediment was extracted further with 2 × 4 ml of chloroform-methanol. The supernatants were combined and an aliquot was taken for the final isolation of the lipids⁷. The lipid-bound phosphorus was analyzed^{8,9} and the ³²P-counts were corrected for decay.

The synthesis of collagen is indicated by the incorporation of ³H-proline to hydroxyproline of granuloma slices. The administration of 40% O₂ in vivo or in vitro during the incubation has approximately additive effects (Figure, a). The effect on the synthesis of non-collagenous protein is much smaller, presumably insignificant (Figure, b).

The effect of oxygen is most conspicuous in the incorporation of labelled cytidine to RNA. The administration of oxygen in vivo during the growth of the granu-

loma is determinative (Figure, c). On the contrary, there is no effect on the synthesis of DNA (Figure, d).

Because the finding on the incorporation of cytidine to RNA suggested that the subcellular system for the synthesis of collagen would be a target of the action of oxygen, the total incorporation of ³²P-phosphate into the lipid fraction was also tested and found increased (Figure, e), but the specific activities of ³²P were not affected.

To conclude, the increased supply of oxygen increases in the experimental granuloma the synthesis of RNA, of extra-nuclear phospholipids and of collagen. The beneficial effect on wound healing depends both on the enhanced formation of the subcellular components and on the stimulation of the collagen synthesis per se.

Zusammenfassung. Ratten, die in 40% Sauerstoffatmosphäre gehalten wurden, wurden experimentelle Granulome mit Viskose-Zellulose Schwämmen s.c. induziert. Die Inkorporation von markiertem Cytidin stieg um 200%, und die Synthese der Phospholipide um 80%. Die Einwirkung der Erhöhung des Sauerstoffgehaltes auf die Kollagensynthese sowohl in vivo als in vitro war additiv und betrug maximal 170%. Weder die Synthese der nichtkollagenen Proteine noch diejenige der Desoxyribonukleinsäuren war wesentlich verändert.

J. NIINIKOSKI and E. KULONEN

Department of Medical Chemistry,
University of Turku,
Turku 3 (Finland), 27 August 1969.

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